THE FREQUENT OCCURRENCE OF MENINGOCOCCI IN THE NASAL CAVITIES OF MENINGITIS PATIENTS AND OF THOSE IN DIRECT CONTACT WITH THEM.*

MARY E. GOODWIN AND ANNA I. VON SHOLLY,
Assistant Bacteriologists, Research Laboratory.

(*From the Research Laboratory of the Department of Health, Dr. William H. Park, Director.*)

EVERYONE familiar with the investigations concerning the etiology of meningitis knows that, owing partly to the difficulty of isolating and keeping alive the meningococcus, partly to its similarity to other micrococci, the work of most investigators has been incomplete and therefore of little permanent value.

As the amount of influence which the results of the investigations here recorded may exert depends largely on the degree to which others are convinced of the thoroughness of the identification of the organisms found in the nasal cavities, it seems best to review briefly the literature in order to see what characteristics the best observers consider as belonging to the meningococcus and therefore as necessary to prove the identity of the suspected organism.

The first important study of the etiology of primary cerebro-spinal meningitis was undertaken by Weichselbaum in 1887. Before that time it had been pretty well established that in secondary cases the pneumococcus was at times the exciting factor, though Leyden[1] and Leichtenstern[2] had noted diplococci in the exudate of fatal cases of primary cerebro-spinal meningitis which they believed to be different from pneumococci. Their descriptions lead one to think that they really saw the meningococcus, but their work was too meager to establish this.

In 1887 Weichselbaum[3] isolated, and carefully studied, cultures from six typical cases of cerebro-spinal meningitis. The cocci had the following cultural characteristics. They grew well on nutrient agar-agar containing 2 per cent of gelatin. The growth on the surface was rather flat and viscid; it was gray in direct, and grayish-white in transmitted, light. The borders were indented and showed the growth to be made up of confluent colonies. Potato showed no visible growth. On the agar-gelatin plates the deep colonies were very small. The surface colonies were grayish white. Under the microscope they were round or irregular, finely granular and their borders indented. They had a golden brown nucleus, an inner light yellow zone, and an outer one which was transparent and colorless. Weichselbaum found it neces-

* Technical portion of Part I of an investigation of cerebro-spinal meningitis carried on under the auspices of the Special Commission of the Department of Health of New York City.

sary to transplant the cultures every two days in order to keep them alive, as they were found to die usually in from three to six days. The cocci themselves were mostly in pairs; some were single and a few in tetrads and small heaps. The single cocci were round, the pairs flattened at the apposed ends. The cocci varied greatly in size and staining, the larger forms, which stained more deeply, being sometimes twice as large as the smaller, more faintly staining ones. They were mostly intracellular in the exudate, and were found only in small numbers in the tissues. All the cultures were Gram negative, and grew well only at blood heat. They did not grow at all at 20° C.

In 1895 H. Jaeger[4] published the results of the study of 14 cultures isolated from typical epidemic cerebro-spinal meningitis. The organisms he isolated differed from those of Weichselbaum and more recent investigators in the following characteristics. There were short chains of four to six elements present in all the cultures, and in two cultures there were long chains of 20 to 30. He describes the cultures as being sometimes Gram positive and sometimes negative. However, he never found Gram positive cocci in the tissues. His cultures grew at lower temperature. The viability of his cultures was much greater, one culture in broth living 43 days. The culture stood drying for 96 days and pus dried on linen gave a growth of the cocci after 127 days. A capsule was present in the smears.

A. Heubner,[5] Jaeger's strongest supporter, describes cultures from four cases which were identical with Jaeger's. Jaeger in his article of 1899[6] still clings to his description of 1895. In 1901[7] he decides that the meningococcus has no capsule, but in other points holds to his original position; nor does he add anything new in his paper of 1903.[12]

Between Jaeger's first and last papers a series of investigations had been carried on which demonstrated to most bacteriologists that either he had failed to isolate the true organisms exciting the disease, or had allowed contaminating or associated bacteria to overgrow and displace the meningococci in his cultures.

Councilman, Mallory, and Wright,[8] after a thorough study of 31 cases, describe their cultures as being similar to those of Weichselbaum.

In 1901 Albrecht and Ghon,[9] after working with 22 cultures, agreed with Weichselbaum. The greater number of cultures observed led them to give wider limits of temperature as suitable for development. Some cultures grew from 25°–40°, though the maximum growth was always between 36°–37°. They are the first to describe the "bread crumb" granules found in the center of the colony after 48 hours. They give the best media as Loeffler's blood serum, or agar containing ascitic fluid. A pellicle on the broth cultures, when the broth was neutral and the cultures were left quiet for several days, was almost constant. In a few instances they kept cultures alive, when protected from drying, for 185 days without transplanting. All the cultures were Gram negative and there was no tendency to chain formation. Albrecht and Ghon obtained cultures from Jaeger and from Heubner and found them not only quite different from theirs, but also unlike each other.

Albrecht and Ghon,[10] and Weichselbaum,[11] in convincing articles published in 1903, take up the peculiarity of Jaeger's cultures point by point, and are of the opinion that he was not working with true meningococcus cultures. Taking the important points agreed upon by the best workers, Albrecht and Ghon give the following characteristics as essential in identifying true meningococcus cultures.

1. Gonococcus-like in form, dividing in the same way, always Gram negative, having many degeneration forms, and often intracellular.

2. Growing only at fairly high temperature, 25°–42°,maximum growth at 36°–37°.

3. Colonies on agar plates luxuriant, quite viscid, glistening, gray in direct light and grayish-white in transmitted light.

4. Growth confined almost entirely to surface in stab culture.

5. Develops pellicle on broth culture (when the broth is neutral and the cultures are undisturbed for several days).

6. Slight pathogenicity for ordinary animals.

7. Non-resistant.

MICROCOCCUS CATARRHALIS.

In 1901 Ghon and H. Pfeiffer[13] published the results of the study of 40 cultures of Micrococcus catarrhalis. They found that, while it grew best on blood agar, it would grow on ordinary media. It differed from the meningococcus in growing more easily, more luxuriantly, and at a lower temperature. The colonies under the microscope were darker, more compact, and had more abrupt margins. Jaeger[14] finds all the strains of M. catarrhalis self-agglutinating. Some of the cultures examined by us have had all the above characteristics, while others have more nearly resembled the meningococcus.

MENINGOCOCCUS CULTURES ISOLATED BY PREVIOUS INVESTIGATORS FROM THE NASAL MUCUS.

In going over the literature we are impressed with the small number of cases from which thoroughly identified meningococcus cultures have been isolated from the nasal mucus. The cases from which Gram negative diplococci closely resembling meningococci have been found in the smears from the nose and throat are, on the contrary, numerous, and have been found by nearly all workers on meningitis.

The first to identify as meningococcus a culture taken from the nasal mucus was F. Kiefer.[15] While working with meningitis cultures he developed a severe purulent rhinitis. The pus contained numerous meningococci. In 1898 Schiff[16] isolated cultures from three out of 29 dispensary patients, a portion of whom suffered from chronic laryngitis, which cultures, he says, Weichselbaum considered true meningococci. These three cultures will be considered later in connection with two obtained by us from medical students, which agreed with the meningococci obtained from the spinal fluid in all respects except in agglutination characteristics. Councilman, Mallory, and Wright[8] report one culture from the throat of a tonsilitis case. Griffon and Gandy[17] twice, at an interval of five days, isolated cultures from the nose of a meningitis case which were identical with cultures from the spinal fluid. Albrecht and Ghon report two instances, one from a case of meningitis, the other from a man whose child died of meningitis three days before the culture was taken. F. Lord,[18] of Boston, isolated meningococci from a case of rhinitis. A. Weichselbaum and Ghon[19] identified one culture from the nose of a meningitis patient and three from the noses of people in contact with patients. These cultures from the 14 cases were the only ones we could find that were studied with sufficient care to warrant their acceptance as true meningococci.

ORIGINAL INVESTIGATION.

Most of the material for this investigation was obtained through the courtesy of Dr. A. W. Taves, of Gouverneur Hospital.

The mucus was taken from the nasal fossæ with a sterile cotton swab and plated out as soon as possible on ascitic agar. As a rule the plates were made within one hour of collecting the specimen, while the swab was still moist. These plates were incubated for from 24 to 48 hours, then fished in the usual way. The colonies were put on blood agar, which seemed to be the most favorable medium.

Several colonies were fished from every type found which resembled a meningococcus colony in color or granularity, and which, under the high power, showed diplococci resembling meningococci. The organisms from the cultures were stained by Gram, and several of the Gram negative ones, which in cultures resembled meningococci, were kept for study.

Fifty-two meningitis cases were examined. Meningococci were isolated from 12 of the 22 cases examined during the first week of the disease, and from 5 of the 15 examined during the second week. In six cases examined during the third week, three during the fourth, and six between the fifth and ninth weeks, no meningococci were found, while in a very severe case examined on the 67th day, we found a few colonies. In one case we failed to get them on the first day and found them in large numbers on the second.

From this it would seem as though the meningococci were present in a rather large percentage of the cases during the first week of the disease.

The nasal secretions of 45 healthy persons living in close contact with meningitis patients were examined. In five of these, meningococci were isolated during the first two weeks of the patients' illness. From the nasal mucus of 55 first year medical students who had never been in known contact with meningitis, there were isolated in two cases a few organisms which were, culturally and in pathogenicity, like meningococci. In studying their agglutination, however, we found that they differed from our other cultures in their specific agglutinins, and therefore were differentiated in one important respect from the latter. In this connection it is of interest that Schiff, in describing his cultures from the nasal cavity of people

not in contact with meningitis, does not refer to agglutination, and evidently did not make the test. His cultures may have differed as ours do. One cannot safely classify these atypical cultures. They may be meningococci derived from a strain different from those isolated by us in the present epidemic, or organisms not capable of readily exciting meningitis, and yet so closely related that they cannot be differentiated without more careful cultural tests than we at present use.

The following tables give the cases, the day of the disease when the specimen was taken, the termination of the disease, and the bacteriological findings.

TABLE 1.

CASES OF MENINGITIS IN WHICH MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Name	Day of Disease	Termination	Percentage of Meningococcus Colonies Present in Plates
W. W.	1	Died	About 55
J. N.	2	Died 3d day	" 90
L. Z.	3	?	" 30
E. R.	3	Died 4th day	" 50
R. T.	3	"	" 40
J. G.	4	?	" 95
Mrs. M.	4	Died 6th day	Very few
S. F.	5	Died	About 50
S. K.	5	?	" 90
J. S.	5	?	" 30
D. M.	7	Recovered	" 2
C. P.	7	?	Few
M.	10	Died	About 10
M. G.	10	"	" 2
J. M.	12	"	A very few
M. H.	14	?	About 95
E. S.	14	Died	" 5
S. K.	67	Died 69th day	" 2

TABLE 2.

CONTACTS WITH MENINGITIS CASES FROM WHOM MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Name	Day of Patients' Illness	Time Since Last Contact	Condition	Percentage of Meningococcus Colonies Present in Plates
Mr. D.	.....	4 days	Normal	About 95
Mrs. D.	.....	4 "	"	" 95
Mrs. K.	14th day	Still in contact	"	" 95
A. K.	14th "	" " "	"	" 50
Mrs. M.	.....	14 days	"	" 30

The plate cultures from the mucus of all these cases contained many colonies and in most cases great numbers of colonies.

TABLE 3

CASES OF MENINGITIS IN WHICH MENINGOCOCCI WERE NOT ISOLATED FROM THE NASAL MUCUS.

No. Examined	Day of Disease	No. Examined	Day of Disease	No. Examined	Day of Disease
1	1	2	13	1	24
1	2	5	14	1	27
2	3	1	15	1	28
5	6	1	17	1	31
1	7	1	18	1	40
1	9	1	19	1	42
1	10	1	20	1	49
1	11	1	21	1	60

TABLE 4.

CONTACTS WITH MENINGITIS CASES FROM WHOM NO MENINGOCOCCI WERE ISOLATED FROM THE NASAL MUCUS.

Number Examined	Days since Contact	Number Examined	Days since Contact
5	2	1	35
9	3	1	50
2	4	3	56
1	10	1	60
1	18	16	Still in contact

All contacts were occupants of the same rooms and nearly always members of the family.

From 14 cases we took multiple specimens. In only one case did we find meningococci in two specimens, 90 per cent on the fifth day, and a very few on the tenth. Table 5 gives the cases, the day of disease, and the bacteriological findings.

CULTURAL CHARACTERISTICS OF THE MENINGOCOCCI ISOLATED FROM THE NASAL MUCUS.

The cultures isolated from the nasal mucus were carried out on the different laboratory media and compared with 30 cultures isolated from a similar number of specimens of spinal fluid.

There were no apparent differences between the nose and spinal fluid cultures. Some grew more luxuriantly than others. The more luxuriant cultures from both spinal fluid and nose seemed to have a more yellow tone, while those growing in a thinner layer were grayish-white.

The morphology of the organisms differed slightly, but the differences were the same for cultures from both sources.

The meningococci occurred as flattened cocci in pairs, fours, and sixes. They varied widely in size in the same culture from the same media, and differed greatly in the intensity with which they took the stain.

In no case did a culture tend to be Gram positive. Cultures were

repeatedly plated out, and numerous colonies fished and stained by Gram. In a culture transplanted twice a day for five days on Loeffler's blood serum, so that the organisms might all be very young; there was no tendency for any of them to be Gram positive.

TABLE 5.

CASES OF MENINGITIS FROM WHICH MULTIPLE SPECIMENS WERE EXAMINED.

Name	Day of Disease	Termination	Findings
B. I.	13	Recovery	No meningococci
	14		" "
	15		" "
	16		" "
E. E.	2	Died 7th day	" "
	3		" "
G. D.	7	Died 45th day	" "
	13		" "
	19		" "
K. S.	5	?	90 per cent
	10		A few
	16		No meningococci
M. D.	7	Recovered	2 per cent meningococci
	13		No meningococci
	19		" "
C. P.	3	?	" "
	4		5 per cent meningococci
	5		No meningococci
	7		" "
	8		" "
	9		" "
S. M.	2	?	" "
	6		" "
	8		" "
S. J.	6	Recovered	" "
	7		" "
	9		" "
	10		" "
S. T.	3	Died 10th day	" "
	4		" "
S. J.	5	?	30 per cent meningococci
	10		No meningococci
	18		" "
W. F.	3		" "
	4		" "
W. J.	17	?	" "
	18		" "
	19		" "
	20		" "
	22		" "
Z. L.	1		" "
	3		30 per cent meningococci

The method of staining by Gram was the same throughout the work and was briefly as follows:

1. Stain two minutes in anilin gentian violet

2. Displace anilin gentian violet with Gram's Iodine Solution and leave on one and one-half minutes.

3. Wash in 95 per cent alcohol until visible color stops coming out.

4. Wash in water and counter stain 30 seconds in watery solution of Bismarck brown (2 grams in 100 c.c.)

In no culture was any tendency to chain formation observed. The cultural characteristics of colonies on ascitic agar plates were as follows:

1. *Macroscopic appearance.*—In many cultures there are two distinct zones, but this was not found constant on repeated plating. Where the colonies are in contact, they are usually divided by a distinct line. They are oval or irregular, grayish-white to yellowish-white, moist and usually viscid, flowing about the needle instead of breaking away from it when they are fished.

2. *Microscopic appearance: Low power.*—Pale amber to brown in color. From fine and evenly granular colonies to those with very coarse central granules. Margins generally rather even and often not abrupt.

3. *Microscopic appearance: High power.*—The diplococci, and occasionally the fours, show plainly. On some plates the margins are smoother and more abrupt, and the separate organisms are distinguished with difficulty.

The most constant characteristics seem to be the coarse central granules and the characteristic separate organisms at the margins when observed with high power.

Ascitic agar slants.—Grayish-white, fairly luxuriant growth, usually with discrete colonies. These colonies at times have a diameter of five millimeters at 48 hours. They are generally quite round, but vary a good deal in the waviness of their outlines. Two zones are often distinguished. In the smears from ascitic agar the organisms stain poorly and are indistinct.

Loeffler's blood serum.—The growth is heavy, moist, confluent and yellowish. The smears show the organisms distinctly, and usually of larger size than on ascitic agar.

Plain agar.—Growth scant, if any, and generally consisting of a few isolated colonies.

Glucose agar.—Growth slightly better than on plain agar.

Glycerin agar.—Same as plain agar.

Blood agar.—Growth very luxuriant, confluent, yellowish-white and extremely sticky; smears same as from Loeffler's.

Sheep serum agar.—Growth fairly luxuriant, about the same as ascitic agar.

Gelatin.—No cultures grew below 24°. At 37° C. all the cultures grew well, with the formation of a heavy pellicle. At the end of six weeks the gelatin still hardened when put in the ice box.

Hiss's inulin medium.—Rendered opaque but not coagulated.

Litmus milk.—The cultures grew only slightly and turned the milk somewhat darker than control at the end of 48 hours, but made no further change.

Marble broth.—Most of the cultures grew slightly, a few grew well, making the medium cloudy, afterward forming a pellicle and sediment. The pellicle was quite general after one week.

Plain broth.—Very few cultures grew in our broth and these only slightly. This was possibly due to an unsuitable reaction of the broth.

Dunham's peptone solution.—Growth very slight. Indol not produced.

Glucose litmus peptone sheep serum agar.—Acid produced after 48 hours.

Lactose litmus peptone sheep serum agar.—Acid produced after 48 hours.

Maltose litmus peptone sheep serum agar.—Acid produced after 48 hours.

Saccharose litmus peptone sheep serum agar.—Acid not produced after 48 hours.

Mannite litmus peptone sheep serum agar.—Acid not produced after 48 hours.

Temperature.—The maximum growth was at about 37°. Nearly all the cultures grew at 30° three months after isolation; a few grew slightly at 24°.

Viability.—The cultures varied greatly in the length of time which they would live without transplanting. In order not to lose cultures we reinoculated them every five days. Many of the cultures on ascitic agar lived from 10 to 20 days without protection from drying, and some of the broth and gelatin cultures lived from five to eight weeks. After 25 cultures were kept in the ice box for five days none of them were alive. Cultures left at room temperature and in the ordinary amount of light varied greatly in their resistance. Most of them failed to grow after 48 hours.

AGGLUTINATION.

Weichselbaum and Ghon[19] and Bettencourt and França[20] found that the serum of meningitis patients agglutinated meningococci in from 1:10 to 1:100 dilutions. They found that the serum of animals immunized for a long time with meningococci agglutinated the cultures only in low dilutions, 1:100 being the highest. We tested the serum of very few patients. The highest dilution agglutinating was 1:200.

Finding it impossible to distinguish between nasal and cord cultures by morphological or cultural comparison, we have made use of a specific serum to aid in classifying the cultures from the different sources.

We inoculated two horses, two sheep, three goats, and 20 rabbits. Only two rabbits lived long enough to give a serum of sufficient agglutinating strength to help in our work. Of these two, one was inoculated with a nasal culture from a student not in contact with meningitis. This serum agglutinated its own culture and several typical meningococcus cultures completely in a dilution of 1:40. The other was inoculated with a cord culture, and agglutinated its own culture in a 1:400 dilution, and other cultures in a 1:50 or slightly higher dilutions.

One sheep, after being inoculated with rather large doses of a cord culture for over three months, gave a serum agglutinating most of the cultures completely in a 1:40 dilution. The goat sera never agglutinated above 1:20.

One horse was inoculated with a nasal culture obtained from a severe case of meningitis on the second day of the disease. The patient died on the third day. This horse died after a month's treatment, before the serum was of much value. The other horse

was given a cord culture, and though he became very sick at the end of the first month, he improved when given smaller doses. At the end of four months the agglutinating strength of this serum was 1:100 for most of our cultures. It seemed better for some other cultures than for its own.

There was a great difference in the degree of agglutinability of the cultures on different days, which made it very difficult to compare the results quantitatively.

The following tables give some of the serum tests with cultures from the spinal fluid and noses of patients, and from the noses of

TABLE 6.

AGGLUTINATION OF 22 CULTURES OBTAINED FROM THE SPINAL FLUID, AND OF 21 FROM THE NASAL MUCUS BY SERUM OF SHEEP 182 AFTER ANIMAL HAD BEEN INOCULATED FOR THREE MONTHS.

	Control	1:20	1:50	1:100	1:200	1:400
33–2 nose	−	+	++	+	+	−
XI–2 nose	−	+	++	+	−	−
124–; cord	−	+	+	±	|	−
VII–3 nose	−	+	+	¦	−	−
W. P. 1 cord	−	+	+	±	−	−
108–5 cord	−	+	+	±	−	−
D. Getz cord	−	+	+	±	|	−
Wiesbard codr	−	+	+	+	−	−
100–2 cord	−	+	+	±	|	−
95–2 nose	−	±	|	−	−	−
140–2 cord	−	+	|	±	−	−
91–1 nose	−	+	+	+	|	−
114–2 nose	−	+¦	±	−	−	−
152–1 cord	−	+	+|	±	|	−
M142–2 nose	−	+	±	±	±	−
Pregalia cord	−	+	+	±	|	−
140–3 cord						
Stolz–2 nose	−	+	±	¦	−	−
Cohen cord	−	±	±	±	¦	−
Fieland cord	−	+	+	+	¦	−
Fieland nose	−	+	+	+	±	−
Goldfarb cord	−	+	+|	+	−	−
Schwartz nose	−	+	+	−	−	−
Goldfarb nose	−	+	±	±	−	−
182 cord	−	++	++	−	−	−
Merrit nose	−	+	±	¦	−	−
136 cord	−	+	+	+	|	|
23–2 nose	−	++	+	−	−	−
IX–2 nose	−	+	−	−	−	−
Horrowitz cord	−	−	−	−	−	−
14 cord	−	++	−	−	−	−
105–5 nose	−	+	+	±	−	−
253–5 cord	−	+	−	−	−	−
36–8 nose	−	++	++	−	−	−
29–3 nose	−	+	+	+	±	|
85–1 nose	−	+	+	|	−	−
142–S cord	−	+	±	−	−	−
Bayridge cord	−	+	−	−	−	−
Rubin nose	−	±	+	±	¦	−
Marzo nose	−	+	+|	±	¦	−
Fielder cord	−	+	+	+	−	−
Gruno cord	−	++	++	+	|	−
McDonald nose	−	±	+	|	−	−

In testing the agglutinating power we used emulsions made from 24 hour sheep serum agar slants in normal salt solution. We used hanging drops, with the slides inverted until the moment of examination, to prevent mistaking mechanical grouping for agglutination. The hanging drops were usually examined after four hours and marked in the following way: − = no agglutination, | = trace, ± = marked trace + = ood agglutination + | = very good agglutination, and ++ = complete agglutination.

contacts and from people not in contact. As a rule, the majority of the cultures seem to agglutinate as well as the culture with which the animal was inoculated.

We saturated the best horse serum with its own culture, with nasal cultures (*a*) from a severe meningitis case, (*b*) from a contact, (*c*) from a non-contact, and with several M. catarrhalis cultures. After allowing the mixture of serum and culture in a 1:5 dilution to stand

TABLE 7.

TESTS OF THE SERUM OF HORSE 277 AFTER BEING INOCULATED FOR FOUR MONTHS WITH 142 S, A SPINAL FLUID CULTURE.

CULTURES	SERUM		SERUM EXTRACTED WITH XI–2, A MENINGOCOCCUS CULTURE FROM THE NASAL MUCUS OF A MENINGITIS PATIENT			SERUM EXTRACTED WITH A M. CATARRHALIS CULTURE FROM A MENINGITIS CASE		
	1:20	1:40	1:5	1:10	1:20	1:5	1:10	1:20
Gruno cord	+	+	–	–	–	+ ł	+	ł
Fielder cord	+	+	–	–	–	+ ł	+	ł
142 S cord	+	+	–	–	–	++	±	±
XI–2 nose	+	+	–	–	–	++	ł	ł
33–2 nose	+	+ ł	–	–	–	++	ł	ł
36–8 nose	±	±	–	–	–	++	–	–
W. nose	+	+	+ ł	ł	ł	+	+	±

These sera after being extracted were in a 1:5 dilution filtered through a Berkefeld filter and the third 10 c.c. used.

for three hours, we filtered through a Berkefeld filter, and used the third 10 c.c. of the filtrate. All the meningococcus-like cultures seemed to remove the agglutinins for all the cultures, while the M. catarrhalis cultures only reduced them about one-third. The control filtration of the serum without exhaustion reduced the agglutinins about as much as did the M. catarrhalis cultures.

TABLE 8.

TESTS OF THE SERUM OF HORSE 277 AFTER BEING INOCULATED FOR FOUR MONTHS WITH 142 S, A SPINAL FLUID CULTURE.

CULTURES	SERUM UNEXTRACTED		SERUM EXTRACTED WITH A SPINAL FLUID CULTURE		SERUM EXTRACTED WITH W. n., A CULTURE FROM A PERSON NOT IN CONTACT WITH MENINGITIS		
	1:100	1:200	1:10	1:20	1:20	1:40	1:100
Gruno cord	+	–	–	–	+	|	–
Fielder cord	–	–	–	–	+	|	–
142 S cord	+	–	–	–	+	|	–
XI–2 nose	+	–	–	–	+	|	–
33–2 nose	+	–	–	–	+	|	–
36–8 nose	+	–	–	–	+	1	–
W. nose	+	–	–	–	–	–	–

The sera, after being extracted, were centrifuged instead of filtered.

We saturated this same horse serum with a meningococcus culture, with a M. catarrhalis culture, and with "W. n." from a student, a non-contact case. Instead of filtering we centrifuged, and found our results somewhat different. The meningococcus culture took out all the agglutinins for the meningitis culture and not for the others, while the M.catarrhalis and the "W. n." left in over half the agglutinins for the meningitis cultures. The M. catarrhalis agglutinated spontaneously, but the non-contact "W. n." took out all of its own agglutinins.

PATHOGENICITY.

Weichselbaum, in 1887, with his original cultures, killed white mice with an intraperitoneal or intrathoracic inoculation of 5 c.c. of a broth dilution of an agar culture or of the water of condensation. The mice died in 36 to 48 hours, and the meningococci were found in the cavity inoculated and usually in the blood. Subcutaneous inoculations were without result. He killed guinea-pigs by inoculating them in the thoracic cavity; but the cocci were not found in the blood or spleen.

Three dogs inoculated subdurally with 1 c.c. and 1.5 c.c. of culture dilution died, one the same evening, the second on the third day, and the third on the 12th day. The first two showed a small amount of fluid blood between the dura and brain. There was a small area of punctiform hemorrhages deeper in the brain, and the membranes were markedly injected. Numerous meningococci were found. In the third dog, between the dura and the right cerebral hemisphere, there was thick red pus, and in the brain a hazel-nut sized abscess containing yellow pus. Around the abscess was a hemorrhagic area. The lateral ventricles contained a red fluid with flakes of pus. No meningococci were found.

Albrecht and Ghon inoculated a goat in the spinal canal. The animal developed symptoms of meningitis and died in five days. The cord showed no changes and meningococci were not isolated.

Our animal work was rather irregular in its results. By inoculating mice intraperitoneally with half of a 24 hour ascitic agar culture of either the cord or nose strains, we caused death in 24 to 48 hours. There was marked congestion of the abdominal viscera, and meningococci were found in the blood and peritoneal exudate.

Rabbits were very uncertain. A few died from subdural inoculation of rather large doses, but there were no typical lesions, and none of them contained meningococci in the blood or exudate.

With small puppies we obtained about the same results as Weichselbaum. When given a dose of two ascitic agar cultures in the spinal canal, the dogs usually died in 24–48 hours. They had convulsions and some rigidity of the neck. On autopsy the membranes were much injected and there were hemorrhagic areas in the cortex. Meningococci were found in these areas, in the fluid under the dura, and in the spinal fluid. As controls to our meningococcus cultures, we used M. catarrhalis cultures and two cultures corresponding culturally to meningococci, which had been isolated from the nasal mucus of normal medical students. The dogs inoculated with two ascitic agar cultures of M. catarrhalis did not die, while those which received the cultures from the students died in 24 hours, and gave the same autopsy results as the dogs inoculated with meningococci.

CONCLUSIONS.

Meningococci were isolated from the nasal mucus of 50 per cent of meningitis patients during the first two weeks of the disease, and from about 10 per cent of the people most closely in contact with them. They were frequently present in enormous numbers.

The two cultures isolated from normal students were like meningococci culturally and in their pathogenicity, but did not have the same specific agglutinins.

The finding of meningococci in great numbers in the nasal mucus of such a large proportion of the patients and of those caring for them, and the absence of meningococci from the nasal mucus of a large number of normal persons examined, would strongly indicate the necessity of isolating cases of epidemic cerebro-spinal meningitis, at least during the early weeks of the disease.

We wish to thank Dr. Park for his constant oversight and direction of our work.

REFERENCES.

1. LEYDEN. *Centralbl. f. klin. Med.*, 1883, 4, p. 161.
2. LEICHTENSTERN. *Deutsche. med. Wchnschr.*, 1885, 11, p. 391.
3. WEICHSELBAUM, A. *Fortschr. der Med.*, 1887, 5, p. 573.
4. JAEGER, H. *Ztschr. f. Hyg.*, 1895, 19, p. 351.

5. HEUBNER, O. *Jahrb. f. Kinderheilkunde*, 1896, 43, p. 1.
6. JAEGER, H. *Dentsche. med. Wchnschr.*, 1889, 25, p. 473.
7. JAEGER, H. *Die cerebrospinal Meningitis als Heeresseuche*, Berlin, 1901.
8. COUNCILMAN, MALLORY, AND WRIGHT. *Special Rep. State Board of Health, Boston, Mass.*, 1898.
9. ALBRECHT AND GHON. *Wien. klin. Wchnschr.*, 1901, 14, p. 984.
10. ALBRECHT AND GHON. *Centralbl. f. Bakt.*, 1903, 33, p. 496.
11. WEICHSELBAUM. *Centralbl. f. Bakt.*, 1903, 33, p. 510.
12. JAEGER, H. *Centralbl. f. Bakt.* 1903, 33, p. 23.
13. GHON AND H. PFEIFFER. *Ztschr. f. klin. Med.*, 1902, 44, p. 262.
14. JAEGER, H. *Ztschr. f. Hyg.*, 1903, 44, p. 225.
15. KIEFER, F. *Berl. klin. Wchnschr.*, 1896, 33, p. 628.
10. SCHIFF. *Centralbl. f. innere Med.*, 1898, 19, p. 577.
17. GRIFFON AND GANDY. *Bull. et mêm. Soc. méd. d. Hôp. de Par.*, 1901, 18 p. 797.
18. LORD, F. *Centralbl. f. Bakt.*, 1903, 34, p. 641.
19. WEICHSELBAUM AND GHON. *Wien. klin. Wchnschr.*, 1905, 18, p. 625.
20. BETTENCOURT AND FRANÇA. *Ztschr. f. Hyg.*, 1904, 46, p. 463.